



## The Impact of Yogurt Enrichment with Millet Milk and Orange Peel Powder

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**Abstract:** In recent years, the development of enriched dairy products with fruits or fruit parts has been growing due to their potential health benefits and consumer preferences. Orange peel is a beneficial source of antioxidants and dietary fiber. People are becoming more conscious of functional essential ingredients in the current lifestyle circumstances, which is driving up demand for functional foods. A significant drought-resistant crop, millet is a nutritionally staple meal throughout Africa and Asia. Additionally, millet is a rich source of bioactive substances that have antioxidant properties. Antioxidants must be consumed through diet if human health is to be improved. This research aimed to evaluate developed yogurt's physiochemical, total phenolic, and total flavonoid content, antioxidant activity, and organoleptic properties. They used cow's milk, barnyard millet milk, and orange peel powder (0.5, 1, 1.5 percent) that had been refrigerated at 4°C for 28 days. As the storage period extended, the pH values of all groups declined, with an increase in titratable acidity. OPPI (Orange Peel Powder) had a high viscosity, but in the syneresis test, it had the lowest value among the enriched yogurt samples. The cell count and pH value of *L.bulgaricus* and *S.thermophilus* decreased after storage, whereas titratable acidity increased. Various methods were used to determine the antioxidant capacity. The antioxidant activity increased in proportion to the quality of the orange peel powder used. Sensory analysis data suggested that among the fortified yogurts, OPPI 0.5% orange peel powder) had the highest overall value during the storage period. As the storage period increased, the organoleptic value decreased. These findings indicate that yogurt fortified with cow's milk, barnyard millet milk, and orange peel powder (0.5, 1, 1.5 percent) has higher quality and antioxidant activity than the control.

**Keywords:** Barnyard Millet Milk, Orange Peel Powder, Ph, Titratable Acidity, Viscosity, Microbial Count, and Antioxidant Activity

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## I. INTRODUCTION

Yogurt is among the most widely consumed fermented milk products globally. Fermented dairy products have gained popularity in recent years among customers, owing to their nutritional advantages and the presence of ingested living microbes. Including barnyard millet milk and fruit peel preparation, which is high in natural antioxidants, may further improve the health benefits of yogurt products<sup>1</sup>. It is developed by the lactic fermentation of two strains: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*<sup>2,3,4</sup>. Massive volumes of fruit waste, mainly peels, seeds, and various other fruit leftovers, are discarded by food processing enterprises<sup>5</sup>. Fruit peels are recycled into various products, including biofuel, agricultural compost, and citric acid<sup>6</sup>. On the other hand, fruit peels are a potential source of carbohydrates, Protein, Fibre, and Phytochemicals phenolic compounds with solid antioxidant capability<sup>7</sup>. These components are rarely collected from peels, representing a potential source of useful antioxidant compounds in the future<sup>8</sup>. The peels of Oranges are high in nutrients and might be utilized as medications or dietary supplements<sup>9</sup>. The antioxidant abilities of plant material owe to the presence of several active phytochemicals, such as vitamins, terpenoids, flavonoids, carotenoids, coumarins, curcumins, saponin, lignin, plant sterol, and so on<sup>10</sup>. Millets are various edible small-seeded types of grass that belong to the Poaceae family (formerly known as Gramineae) and are farmed in semi-arid and arid locations across the world<sup>11</sup>. Millets are a good source of nutrients and give significant health advantages in gluten-free and multigrain cereal products<sup>12</sup>. Yogurt acts as a probiotic carrier food that is considered easy to incorporate probiotics, resulting in high probiotic viability. Bio-yogurt is considered an ideal source for the delivery of viable probiotic strains, *L. acidophilus* and *Bifidobacterium bifidum*, the most common probiotics used in the dairy industry. However, to attain the probiotic effect, it is reported that the need to consume adequate amounts of viable probiotic cells regularly is known as the therapeutic minimum. Therefore, the consumption should be more than 100 g of bio-yogurt containing more than 10<sup>6</sup> cfu mL<sup>-1</sup> viable cells<sup>13</sup>. Consumption of probiotics seems to be helpful in maintaining good health, restore body vigor and combat intestinal disorders through the therapeutic and beneficial effects associated with them. Probiotics are reported to have therapeutic effects such as preventing urogenital infections, alleviation of constipation, protection against diarrhea, infant diarrhea, prevention of hypercholesterolemia, protection against colon/bladder cancer, and prevention of osteoporosis. On the other hand, probiotics are claimed to have other beneficial effects such as maintenance of normal intestinal flora, enhancement of the immune system, reduction of the lactose-intolerance and serum cholesterol levels, and enhanced anticarcinogenic activity<sup>13-15</sup>. Moreover, yogurt is reported to be beneficial for the treatment of Inflammatory Bowel Disease (IBD), including gastrointestinal disorders such

as Crohn's disease, ulcerative colitis and pouchitis. The VSL#3 (a mixture of four strains of lactobacilli including *L. casei*, *L. Plantarum*, *L. acidophilus* and *L. delbrueckii ssp. bulgaricus*, three strains of bifidobacteria including *B. longum*, *B. breve* and *B. infantis* and one strain of *S. thermophilus*) were found to be effective in maintaining remission in patients with chronic relapsing pouchitis<sup>16</sup> and for the prevention of pouchitis in patients who had ileo-pouch anal anastomosis for ulcerative colitis<sup>14,17</sup>. On the other hand, Ishikawa et al.<sup>18</sup> reported that the supplementation of Bifidobacteria fermented milk for 1 year was successful in maintaining remission and claimed beneficial preventive effects on the relapse of ulcerative colitis. Therefore, the current research was to determine the fortified yogurt's physiochemical, antioxidant, and organoleptic properties.

## 2. MATERIALS AND METHODS

### 2.1 Raw Materials and Starter Cultures

For the preparation of yogurt, fresh cow's milk was purchased from the nearby Aavin parlour (A unit of Tamil Nadu Co-operative Milk Producers Federations Limited India), which is located inside Periyar University. Lactina Starter Culture with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, purchased from Yogurt bio.

### 2.2 Yogurt Production and Sample Collection

#### 2.2.1 Preparation of Orange Peel Powder

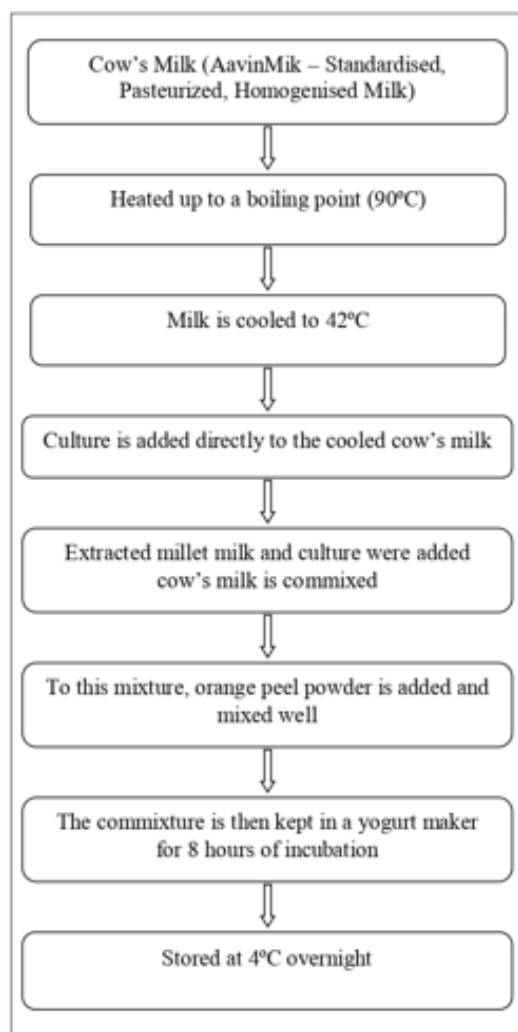
Orange was acquired in bulk from the fruit market of Salem. Orange was washed thoroughly, peeled, and the fruit peels were chopped into small pieces and allowed to dry in a tray-dryer at 60°C - 70°C for 24 – 48 hours. The dried peel was converted into fine powder form and sieved, then packed in an airtight container for further use.

#### 2.2.2 Preparation Millet Milk

Barnyard millet was acquired in Salem's local market. Dust, broken seeds, and other foreign objects were manually removed from millet. After that, the millet was steeped in water overnight for 16 hours. The soaked millet was then grinded in a wet grinder with addition of water. Using muslin fabric, the milk was collected from the millet that had been ground. Before combining with cow's milk, the extracted millet milk was heated to 80 – 85 degrees Celsius and then cooled to 42 degrees Celsius.

#### 2.2.3 Preparation of Yogurt

The schematic representation of the steps involved in the orange peel powder incorporated yogurt is shown below (Figure 1)



**Fig 1: Processing steps of yogurt**

## 2.3 Physicochemical Analysis of Yogurt

### 2.3.1 pH and Titratable Acidity

The measurement of pH was carried out with a digital pH meter (Testo 206 pH2 I-Kit). To determine titratable acidity, 10 mL of yogurt was titrated with 0.1 M sodium hydroxide solution. The titratable acidity was expressed as a gram of lactic acid/100 g of yogurt and was calculated using the following equation:

$$\text{Total acidity} = \frac{V \times \text{NaOH factor} \times A \times D}{\text{Volume of Sample}} \times 100$$

V is the volume of NaOH added (mL), A is the conversion factor (0.009 for lactic acid), D is the dilution factor, and F is a factor of 0.1 N NaOH<sup>19</sup>.

### 2.3.2 Viscosity

The viscosity of the yogurt sample was determined using a FungilabViscolead rotational viscometer with a spindle L4. The reading was taken in triplicates at 3rpm rotation speed—results recorded in centipoises (cP)<sup>20</sup>.

### 2.3.3 Evaluation of Colour

Colour parameters were determined with a tintometer (Lovibindcolour measurement tintometer group LC100 SV100 Kit), with illuminated D65 as a reference: L\* (100 = White; 0 = Black), a\* (+red; -green), b\* (+yellow; - blue). The instrument was calibrated before starting the evaluation of sample color by closing the lid, and then the samples were subjected to analysis in triplicate values.<sup>20</sup>

### 2.3.4 Syneresis

For syneresis, 10ml of yogurt was centrifuged at 1500 RPM for twelve minutes at 4°C and separated what was measured. The rate of syneresis was calculated using the following equation:<sup>20</sup>

$$\text{Syneresis (\%)} = \frac{(W_s)}{(W_y)} \times 100$$

Where W<sub>s</sub> = the supernatant after centrifugation  
W<sub>y</sub> = the yogurt in a tube

The analyses were performed in triplicate.

## 2.4 Microbiological Analyses

Plating and isolation were done following the procedures. Enumeration was using pour plate technique. *S. thermophilus* and *L. bulgaricus* were enumerated on M17 and MRS agar, respectively. Serial dilutions were prepared using peptone diluents. One ml of thoroughly mixed yogurt sample was transferred sterile 1 ml pipette to the first tube of 9 ml sterile diluent, representing the 10<sup>-1</sup> dilution. The diluted sample was blended for one minute by a vortex mixer. Next, one ml of 10<sup>-1</sup> dilution was transferred to the second tube of 9 ml sterile diluent 10<sup>-2</sup> dilution. This operation was repeated until dilution was obtained by using fresh and sterile pipettes and diluents. For counting of *L. bulgaricus*, one ml of dilution was transferred into the Petri dishes in triplicates then 12 ml of MRS agar medium<sup>21</sup> at 45°C was poured into each Petri dish with dilution. The content was mixed carefully by rotating the five times clockwise and five times counter-clockwise, then allowed to solidify on a level surface. Plates were inverted and incubated anaerobically in a tightly sealed anaerobic jar at 37°C for 72 hours. For counting *S. thermophilus*, diluents were used for preparing serial dilutions. One ml of appropriate dilution was transferred into in triplicates then 12 to 15 ml of M17 agar at 45°C was added into each containing one ml of appropriate dilution. The content was mixed carefully by rotating the Petri dish five (5) times clockwise and five (5) times counter-clockwise, then allowed to solidify on the surface. Plates were then inverted and incubated aerobically at 37°C for 48 hours. Colonies in a plate with 25-250 colonies were counted, and viable count in CFU/ml was calculated.

$$N = \sum C / [(1.0 * n_1) + (0.1 * n_2)] * d$$

Where;

N= number of colonies ml or gram of sample.

∑C=sum of all the settlements in all plates counted.

Number of plates in the lower dilution counted.

n<sub>2</sub>=number of plates in the subsequent higher dilution counted.

d=dilution from which the first counts were obtained.

## 2.5 Total Phenolic Content (TPC)

The measurement of TPC was performed as described by Hernandez-Carranza et al.<sup>22</sup>. In brief, in an amber glass tube, 1 mL of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (0.1N); after 3 minutes, 1 mL of NaCO<sub>3</sub> (0.05%) was added and stored for 30 minutes at room temperature in a dark environment. The number of total phenols was measured as mg of gallic acid equivalent (GAE)/100g of dry weight using a UV-vis spectrophotometer calibrated to 765nm.

## 2.6 Total Flavonoid Count Content

According to Hernández-Carranza et al.,<sup>22</sup>, the TFC was assessed. Before standing for 10 minutes, 500μL of each sample was combined with 500μL of NaNO<sub>2</sub> (1.5%, 500 μL) in a vortex. After adding the AlCl<sub>3</sub> (3%), 1000 mL of NaOH 1N was added, stirred for 2 minutes, and read at 490 nm. The quercetin standard curve was then run. The outcome was given as milligrams of quercetin per 100 grams of yogurt.

## 2.7 Antioxidant Activity

### 2.7.1 ABTS

ABTS radical scavenger activity of yogurt samples was

determined using the method of Re et al.<sup>23</sup>. Minor corrections. First, ABTS was dissolved in distilled water to a concentration of 7 mM. ABTS radical cations were prepared by adding ABTS stock solution to 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (2:1 ratio). Cover with aluminum foil and store in the dark for 24 hours before use. ABTS reagent was diluted in 94% ethanol to the appropriate absorbance (0.17±0.03) measured at 734 nm. ABTS reagent (950 μl) was mixed with 50 μl of the test sample at the indicated concentration. The mixture was covered with aluminum foil and left in the dark for 10 minutes at room temperature. Absorbance at 734 nm was recorded with a microplate reader. Each sample was measured in triplicate, and percent inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100$$

### 2.7.2 FRAP

Ferric reducing antioxidant power (FRAP) describes the ability of the analyzed substance to reduce the complex of Fe (III)–2,4,6-tris(2-pyridyl)-s-triazine to the form of Fe (II)–TPTZ. The intensity of the blue color, measured spectrophotometrically at 583 nm using a Rayleigh UV-1601 spectrophotometer (Beijing Rayleigh Analytical Instruments, Beijing, China), is linearly correlated with the reducing agent concentration. Antioxidant power is expressed as mM of Fe<sup>2+</sup> per l L, based on a standard curve  $y = 0.0001x + 0.0113$  ( $r^2 = 0.9938$ ); where y is absorbance and x is standard (Fe II) or evaluated sample concentration.

### 2.7.3 DPPH Assay

The evaluation of DPPH scavenging ability was performed by mixing 0.5ml of the sample with 2.6mg of 0.066mM DPPH solution, in a UV-VIS spectrophotometer. The absorbance was recorded at 516nm after 30 minutes' reaction at 37°C. The percentage of DPPH scavenging of the yogurt was calculated using to the equation:

$$\text{DPPH Scavenging percentage (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} * 100$$

Where A control = Absorbance of DPPH radical + Methanol  
A sample = Absorbance of DPPH radical + yogurt sample

## 2.8 Organoleptic Evaluation

The yogurt samples were evaluated organoleptically on the first day after manufacturing (at 4°C). Samples were subjected to evaluation by 10 untrained panelists who are members of Periyar University (Salem, Tamil Nadu). Each item of the evaluation was given a score on the 9-point hedonic scale: liked extremely = 9, liked very much = 8, liked moderately = 7, liked slightly = 6, neither liked nor disliked = 5, disliked slightly = 4, disliked moderately = 3, disliked very much = 2, and disliked extremely = 1. In addition, color, aroma, mouthfeel, consistency, taste, flavor and overall acceptability were all assessed.

## 2.9 Statistical Analysis

The Data were recorded using M.S. excel and analyzed using SPSS version 16. Proportions were recorded and continued data were reported as mean ± standard deviation of the mean. ANOVA (Analysis of variance) was used to compare mean

values within and between groups, and the mean separation was obtained using the Duncan procedure. The statistical significance of the data was indicated at a P value < 0.05.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. pH, Titratable acidity and microbial analysis

During the storage period, the variation in pH, Titratable acidity, and total lactic acid bacteria counts (*L.bulgaricus* and *S.thermophilus*) of yogurts (Table 1). The pH of the sample was 4.64 to 4.26 at the start of the storage, but it dropped to 4.31 to 4.06 after 28 days. As storage time increased, pH declined in all samples ( $p < 0.05$ ). During the period of storage, lactose fermentation reduced the pH<sup>24</sup>. This could be due to the bacteria's metabolic activity, increasing the transformation of lactose to lactic acid<sup>25</sup>. In general, it has been reported that the ideal pH range for thick fermented milk entering the market is 3.27 to 4.69<sup>26</sup>. In this investigation, yogurt that had been refrigerated for 28 days had a pH within this range, without any difference in quality, compared to fermented milk from the market. Yogurt's initial titratable acidity (Table 1) ranged from 0.73 to 1.25%. The value of titratable acidity increased from 0.88 to 1.32% after 28 days. The storage period continued with an increase in the titratable acidity value. The amount of non-fat solid substances like proteins, citrates, and phosphates affect titratable acidity<sup>27</sup>. Previous research reported a decline in the pH value while a rise in titratable acidity because of the

generation of acid, identical to our findings<sup>28</sup>. Because of the higher availability of carbohydrate sources from the fruit peel and barnyard millet milk to the metabolic activity of both yogurt cultures (*L.bulgaricus* and *S.thermophilus*) resulting from a higher level of organic acids, the yogurt sample demonstrated a steeper decrease in Ph and concurrent increase of acidity (Table 1). Similar pH alterations for control yogurt and yogurt incorporating pineapple peel powder were found in a prior investigation<sup>29</sup>. Additionally, titratable acidity in yogurt containing passion fruit peel powder was higher than their respective control yogurts<sup>30</sup>. Microbial characteristics were assessed by counting the viable lactic acid bacteria cells of. For 28 days, the viable cell counts of *L.bulgaricus* and *S.thermophilus* were determined in yogurts supplemented with barnyard millet milk and orange peel powder. (Table 1) presents the differences in lactic acid bacteria in yogurt samples. The microbial counts of *L.bulgaricus* and *S.thermophilus* on the first day of storage were significantly different ( $p < 0.05$ ), ranging from 7.83 to 8.65 Log CFU/g and 7.54 to 9.20 Log CFU/g, respectively. The viable bacterial counts (*L.bulgaricus* and *S.thermophilus*) increased until day 7 and then began to decline for all yogurt samples during storage, ranging from 7.50 to 8.05 Log CFU/g and 7.32 to 8.58 Log CFU/g on 28<sup>th</sup> day. Lactic acid bacteria count in all yogurt samples exceeded the Codex minimum threshold of 7.0 Log CFU/g. The addition of barnyard millet milk and orange peel powder to yogurt did not adversely influence the growth of LAB.

**Table 1: pH, Titratable acidity and lactic acid bacteria count of prepared yogurts with millet milk and orange peel powder**

	Storage Period	CM	CM + MM	OPPI	OPP2	OPP3
pH	1 <sup>st</sup> Day	4.26 ± 0.05 <sup>aB</sup>	4.64 ± 0.02 <sup>cE</sup>	4.40 ± 0.03 <sup>bB</sup>	4.40 ± 0.00 <sup>bB</sup>	4.44 ± 0.02 <sup>bD</sup>
	7 <sup>th</sup> Day	4.26 ± 0.09 <sup>aB</sup>	4.56 ± 0.02 <sup>cD</sup>	4.83 ± 0.08 <sup>dC</sup>	4.44 ± 0.01 <sup>bB</sup>	4.46 ± 0.02 <sup>bD</sup>
	14 <sup>th</sup> Day	4.24 ± 0.06 <sup>aB</sup>	4.46 ± 0.02 <sup>bC</sup>	4.45 ± 0.03 <sup>bB</sup>	4.40 ± 0.15 <sup>bB</sup>	4.25 ± 0.00 <sup>aC</sup>
	21 <sup>st</sup> Day	4.09 ± 0.03 <sup>aA</sup>	4.36 ± 0.03 <sup>cdB</sup>	4.39 ± 0.01 <sup>dB</sup>	4.32 ± 0.02 <sup>cAB</sup>	4.15 ± 0.03 <sup>bB</sup>
	28 <sup>th</sup> Day	4.06 ± 0.02 <sup>aA</sup>	4.30 ± 0.01 <sup>cA</sup>	4.31 ± 0.01 <sup>cA</sup>	4.26 ± 0.03 <sup>bA</sup>	4.09 ± 0.03 <sup>aA</sup>
Titratable Acidity	1 <sup>st</sup> Day	0.74 ± 0.03 <sup>aA</sup>	0.73 ± 0.02 <sup>aA</sup>	1.25 ± 0.01 <sup>dB</sup>	1.17 ± 0.02 <sup>cB</sup>	1.10 ± 0.00 <sup>bA</sup>
	7 <sup>th</sup> Day	0.77 ± 0.03 <sup>aAB</sup>	0.78 ± 0.01 <sup>aB</sup>	1.23 ± 0.01 <sup>dA</sup>	1.15 ± 0.01 <sup>cAB</sup>	1.08 ± 0.01 <sup>bA</sup>
	14 <sup>th</sup> Day	0.79 ± 0.02 <sup>aB</sup>	0.83 ± 0.02 <sup>bC</sup>	1.29 ± 0.01 <sup>dC</sup>	1.14 ± 0.01 <sup>cA</sup>	1.15 ± 0.02 <sup>cB</sup>
	21 <sup>st</sup> Day	0.83 ± 0.01 <sup>aC</sup>	0.86 ± 0.02 <sup>bD</sup>	1.29 ± 0.01 <sup>eC</sup>	1.19 ± 0.01 <sup>dC</sup>	1.16 ± 0.01 <sup>cB</sup>
	28 <sup>th</sup> Day	0.88 ± 0.01 <sup>aD</sup>	0.89 ± 0.01 <sup>aE</sup>	1.32 ± 0.02 <sup>dD</sup>	1.22 ± 0.01 <sup>cD</sup>	1.19 ± 0.01 <sup>bC</sup>
<i>L. bulgaricus</i> (log CFU/g)	1 <sup>st</sup> Day	8.65 ± 0.01 <sup>dD</sup>	8.15 ± 0.01 <sup>cC</sup>	7.89 ± 0.01 <sup>bD</sup>	7.83 ± 0.01 <sup>aD</sup>	7.84 ± 0.01 <sup>aD</sup>
	7 <sup>th</sup> Day	8.75 ± 0.01 <sup>eE</sup>	8.35 ± 0.01 <sup>dE</sup>	7.95 ± 0.01 <sup>cE</sup>	7.87 ± 0.01 <sup>aE</sup>	7.88 ± 0.01 <sup>bE</sup>
	14 <sup>th</sup> Day	8.45 ± 0.01 <sup>eC</sup>	8.25 ± 0.01 <sup>dD</sup>	7.84 ± 0.01 <sup>cC</sup>	7.78 ± 0.01 <sup>bC</sup>	7.76 ± 0.01 <sup>aC</sup>
	21 <sup>st</sup> Day	8.25 ± 0.01 <sup>eB</sup>	8.08 ± 0.01 <sup>dB</sup>	7.78 ± 0.01 <sup>cB</sup>	7.63 ± 0.01 <sup>aB</sup>	7.69 ± 0.01 <sup>bB</sup>
	28 <sup>th</sup> Day	8.05 ± 0.01 <sup>eA</sup>	7.84 ± 0.01 <sup>dA</sup>	7.63 ± 0.01 <sup>cA</sup>	7.50 ± 0.01 <sup>aA</sup>	7.61 ± 0.01 <sup>bA</sup>
<i>S. thermophilus</i> (log CFU/g)	1 <sup>st</sup> Day	9.20 ± 0.01 <sup>eD</sup>	8.57 ± 0.01 <sup>dC</sup>	7.54 ± 0.01 <sup>aC</sup>	7.81 ± 0.01 <sup>cD</sup>	7.64 ± 0.01 <sup>bD</sup>
	7 <sup>th</sup> Day	9.28 ± 0.01 <sup>eE</sup>	8.72 ± 0.01 <sup>dE</sup>	7.62 ± 0.01 <sup>aE</sup>	7.89 ± 0.01 <sup>cE</sup>	7.78 ± 0.01 <sup>bD</sup>
	14 <sup>th</sup> Day	9.03 ± 0.01 <sup>eC</sup>	8.61 ± 0.01 <sup>dD</sup>	7.58 ± 0.01 <sup>aD</sup>	7.76 ± 0.01 <sup>cC</sup>	7.63 ± 0.01 <sup>bC</sup>
	21 <sup>st</sup> Day	8.77 ± 0.01 <sup>eB</sup>	8.36 ± 0.01 <sup>dB</sup>	7.44 ± 0.01 <sup>aB</sup>	7.63 ± 0.01 <sup>cB</sup>	7.58 ± 0.01 <sup>bB</sup>
	28 <sup>th</sup> Day	8.58 ± 0.01 <sup>eA</sup>	8.29 ± 0.01 <sup>dA</sup>	7.32 ± 0.02 <sup>aA</sup>	7.51 ± 0.01 <sup>cA</sup>	7.42 ± 0.01 <sup>bA</sup>

<sup>a-d</sup> Means in the same row followed by different lower-case letters represent significant differences by barnyard millet milk and orange peel powder ( $p < 0.05$ ). <sup>A-E</sup> Mean in the same column followed by other lower-case letters represents significant difference by period ( $p < 0.05$ ). CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPPI: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder.

#### 3.2. Viscosity and Syneresis

The results of syneresis and viscosity of yogurt samples refrigerated at 4°C for 28 days are presented (Table 2). Except

for OPPI, without any significant difference ( $p > 0.05$ ), the viscosity of all samples reduced over the long storage period, but with a significant difference ( $p < 0.05$ ). It was observed that adding orange peel powder decreased viscosity values. This could be due to the influence of orange peel powder on the electrostatic aggregation of casein network in yogurts and the resistance of the yogurt matrix to flow. It was also stated in a previous study that, the incorporation of plant extract often affected the consistency of dairy products due to the lower water-binding ability of its proteins<sup>31</sup>. As the storage period was extended, syneresis tended to rise in all groups ( $p < 0.05$ ). acidity directly affects syneresis, and pH has an inverse correlation with it<sup>32</sup>. By gradually dissolving calcium and inorganic phosphate, acidification diminishes the net negative electric charge of casein micelles. Casein approaches the

isoelectric point when the pH is reduced (especially below 4.6), and electrostatic repulsions are reduced by promoting protein-to-protein interactions<sup>33,34</sup>. Even a little drop in pH lowers the electric charge, lowering colloid stability<sup>35</sup>. Physical

qualities influence whey separation during storage, which can be avoided by increasing the total solid content of the additional stabilizer<sup>36</sup>.

**Table 2: Viscosity and Syneresis of prepared yogurts with millet milk and orange peel powder**

	Storage Period	CM	CM + MM	OPPI	OPP2	OPP3
Viscosity (cp)	1 <sup>st</sup> Day	14968 ± 810.59 <sup>eC</sup>	13150 ± 601.75 <sup>dD</sup>	10081 ± 2.65 <sup>aA</sup>	11975 ± 5.03 <sup>cE</sup>	11033 ± 2.64 <sup>bE</sup>
	7 <sup>th</sup> Day	13834 ± 539.82 <sup>eB</sup>	12562 ± 420.15 <sup>dCD</sup>	10005 ± 2.64 <sup>aA</sup>	11871 ± 23.89 <sup>cD</sup>	11003 ± 4.62 <sup>bD</sup>
	14 <sup>th</sup> Day	12638 ± 332.15 <sup>dA</sup>	11868 ± 815.96 <sup>cBC</sup>	9994.3 ± 3.22 <sup>aA</sup>	11813 ± 63.84 <sup>cC</sup>	10978 ± 17.04 <sup>bC</sup>
	21 <sup>st</sup> Day	12245 ± 303.22 <sup>dA</sup>	11355 ± 605.35 <sup>bcAB</sup>	9863.7 ± 33.50 <sup>aA</sup>	11685 ± 10.51 <sup>cB</sup>	10886 ± 15.27 <sup>bb</sup>
	28 <sup>th</sup> Day	12069 ± 102.65 <sup>dA</sup>	10507 ± 170.67 <sup>bA</sup>	9181.3 ± 10.33 <sup>aA</sup>	11605 ± 5.51 <sup>cdA</sup>	10771 ± 17.16 <sup>bCA</sup>
Syneresis (%)	1 <sup>st</sup> Day	29.83 ± 0.76 <sup>aA</sup>	50.67 ± 0.76 <sup>bA</sup>	77.33 ± 0.29 <sup>dA</sup>	75.17 ± 0.29 <sup>cA</sup>	77.50 ± 0.00 <sup>dA</sup>
	7 <sup>th</sup> Day	30.17 ± 0.29 <sup>aA</sup>	51.33 ± 0.29 <sup>bAB</sup>	77.83 ± 0.76 <sup>dAB</sup>	76.17 ± 0.29 <sup>cB</sup>	78.17 ± 0.29 <sup>dB</sup>
	14 <sup>th</sup> Day	31.83 ± 0.76 <sup>aB</sup>	52.17 ± 0.58 <sup>bB</sup>	78.33 ± 0.29 <sup>dB</sup>	77.33 ± 0.29 <sup>cC</sup>	78.50 ± 0.00 <sup>dB</sup>
	21 <sup>st</sup> Day	33.00 ± 0.50 <sup>aC</sup>	55.00 ± 1.00 <sup>bC</sup>	78.83 ± 0.29 <sup>cdCD</sup>	78.00 ± 0.50 <sup>cD</sup>	79.33 ± 0.29 <sup>dC</sup>
	28 <sup>th</sup> Day	35.00 ± 1.00 <sup>aD</sup>	59.00 ± 0.87 <sup>bD</sup>	79.50 ± 0.00 <sup>cdD</sup>	78.33 ± 0.29 <sup>cD</sup>	80.00 ± 0.50 <sup>dD</sup>

<sup>a-d</sup> Means in the same row followed by different lower-case letters represent significant differences by barnyard millet milk and orange peel powder ( $p < 0.05$ ). <sup>A-E</sup> Mean in the same column followed by different lower-case letters represents significant difference by period ( $p < 0.05$ ). CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPPI: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder

### 3.3. Color Evaluation

The  $L^*$ ,  $a^*$  and  $b^*$  values of yogurt that was refrigerated at 4°C for 28 days is presented (Table 3). Transitions from dark to lighter shades, greenness to reddish variants, and blueness to yellowness are indicated by the  $L^*$ ,  $a^*$  and  $b^*$  values, respectively. When the concentration of orange peel powder was increased, the value was highest in the order CM > CM + MM > OPPI > OPP2 > OPP3, while  $a^*$  and  $b^*$  values increased from 0.43 to 1.47 and 9.07 to 17.33, respectively (0.5, 1 and 1.5 percent). The value of  $L^*$  declined as the storage period was prolonged, but the value of  $a^*$  and  $b^*$  rose. Thus, it can be

concluded that adding barnyard millet milk and orange peel powder changed the color of the yogurt.  $L^*$  value is an estimation of food whiteness. Whiteness in fluid milk results from colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum. Previous reports have shown that consumers have the highest appeal for fluid milks with visual properties characteristic of whole milk, and the perception of milk whiteness has been demonstrated to have the most positive influence on increasing consumer appeal. It also needs to be mentioned that milk is a fine food and as fermentation goes on it loses clarity. No significant differences ( $P < 0.05$ ) in color parameters  $L^*$  values started to decline and  $a^*$  and  $b^*$  values started to increase. good correlation coefficients have been found ( $P < 0.01$ ),  $b^*$  parameter is the one with the best relation coefficient ( $R$ )<sup>37</sup>. Fruit peel powder addition imparted the changes in color values. In one of the previous studies, yogurt containing 0.6%, 0.8%, and 1% orange peel powder had more red and yellow color than the control, while in another study, it has found that the incorporation of powder obtained from asparagus shoots imparted a yellowish-greenish color to the yogurt. Similarly, in the present study, lightness decreased and redness and yellowness increased with the fiber addition.<sup>37</sup>

**Table 3: Color values of prepared yogurt with millet milk and orange peel powder**

	Storage Period	CM	CM + MM	OPPI	OPP2	OPP3	Sig		
							Y	D	Y × D
$L^*$	1 <sup>st</sup> Day	83.63 ± 0.57d	80.23 ± 0.06c	73.77 ± 1.00b	72.77 ± 0.47ab	72.50 ± 0.72a	***	***	***
	7 <sup>th</sup> Day	82.80 ± 0.60e	80.57 ± 0.32d	75.37 ± 1.08c	73.70 ± 0.35b	71.83 ± 0.38a	***	***	***
	14 <sup>th</sup> Day	82.23 ± 0.93d	78.00 ± 0.10c	76.27 ± 0.35b	77.40 ± 0.36bc	72.53 ± 1.00a	***	***	***
	21 <sup>st</sup> Day	81.13 ± 0.12d	77.97 ± 0.93c	80.40 ± 0.80d	73.20 ± 0.17b	69.13 ± 1.97a	***	***	***
	28 <sup>th</sup> Day	81.80 ± 0.44d	75.67 ± 1.02c	77.77 ± 2.29c	73.20 ± 0.75b	67.00 ± 1.25a	***	***	***
$a^*$	1 <sup>st</sup> Day	0.43 ± 0.15a	1.47 ± 0.06c	0.90 ± 0.00b	0.97 ± 0.06b	1.00 ± 0.10b	***	***	***
	7 <sup>th</sup> Day	0.37 ± 0.23a	1.63 ± 0.12d	0.70 ± 0.10b	1.17 ± 0.06c	1.53 ± 0.12d	***	***	***
	14 <sup>th</sup> Day	0.77 ± 0.15b	1.87 ± 0.06d	0.20 ± 0.35a	0.17 ± 0.21a	1.17 ± 0.06c	***	***	***
	21 <sup>st</sup> Day	0.67 ± 0.12b	2.00 ± 0.10d	-0.10 ± 0.20a	1.03 ± 0.15c	1.80 ± 0.20d	***	***	***
	28 <sup>th</sup> Day	0.80 ± 0.35a	2.20 ± 0.00b	0.53 ± 0.38a	1.00 ± 0.10a	2.60 ± 0.26b	***	***	***
$b^*$	1 <sup>st</sup> Day	9.07 ± 0.12a	9.43 ± 0.06a	12.53 ± 0.32b	15.07 ± 0.29c	17.33 ± 0.15d	***	***	***
	7 <sup>th</sup> Day	6.60 ± 0.10a	10.27 ± 0.67b	13.10 ± 0.26c	16.33 ± 0.47d	18.30 ± 0.00e	***	***	***
	14 <sup>th</sup> Day	9.23 ± 0.59a	16.17 ± 0.06c	13.30 ± 0.17b	17.37 ± 0.15d	19.03 ± 0.45e	***	***	***
	21 <sup>st</sup> Day	9.17 ± 0.32a	15.77 ± 0.23b	14.17 ± 0.06b	14.53 ± 1.86b	16.43 ± 1.80b	***	***	***
	28 <sup>th</sup> Day	9.63 ± 0.49a	15.70 ± 0.66bc	12.53 ± 1.37ab	16.37 ± 1.33c	12.33 ± 3.99ab	***	***	***

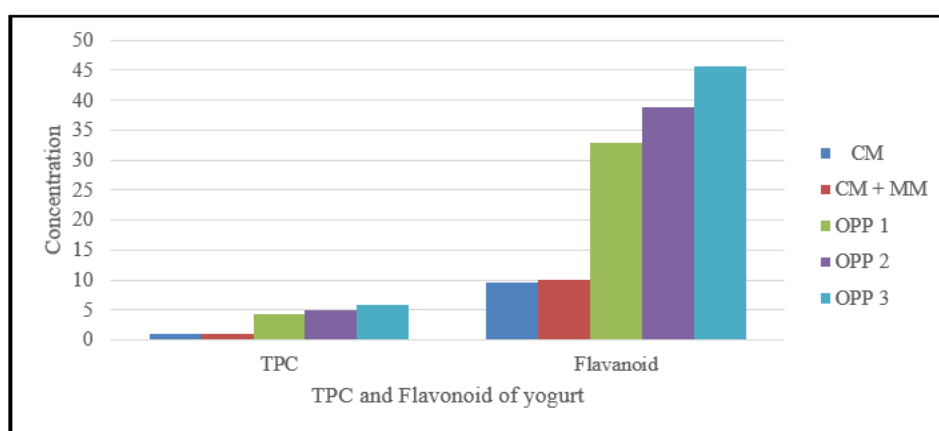


<sup>a-d</sup> Means in the same row followed by different lower-case letters represent significant difference by barnyard millet milk and orange peel powder ( $p < 0.05$ ). CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPP1: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder. Y: Yogurt; D: Storage days; \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.00$ .

### 3.4. Determination of Total Phenolic Content (TPC) and Flavonoid content

Results for the TPC of yogurt samples are illustrated (Figure 2). In comparison to CM yogurt, all types of yogurts had significantly higher TPC ( $p < 0.05$ ). the higher TPC in CM yogurt is likely a result of the availability of polyphenols in milk, which are largely derived from feed<sup>38</sup> protein, and reducing components<sup>39</sup>. OPP3 seemed to have the highest phenolic concentration, with  $5.87 \pm 0.01$  mg GAE/100g of yogurt. The

TPC rose as the amount of orange peel powder made with barnyard millet milk and cow's milk increased. An earlier study found that yogurt enriched with callus and grape extract had a higher TPC value<sup>40</sup>. In another investigation, yogurt with grape seed extracts had a higher TPC value<sup>39</sup>. The result showed a high amount of flavonoid contain variation OOP3 ( $45.64 \pm 0.76$ ). the lowest of amount of flavonoid found CM variation it contained ( $9.56 \pm 0.56$ ). Flavonoid is bioactive phenols commonly found in fruits, vegetables, and parts of plants. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants. Fermentation increases antioxidant activity and, thus, the functional value of the foodstuff. Polyphenolic flavonoids display strong antioxidant activity. The flavonoid and lactic acid bacteria content in yogurt gives it potential as a functional food product. Flavonoids have antioxidant activity that can help protect against free radicals and prevent clinical complications from metabolic diseases, such as diabetes mellitus, dyslipidemia, and metabolic syndrome.<sup>41</sup>



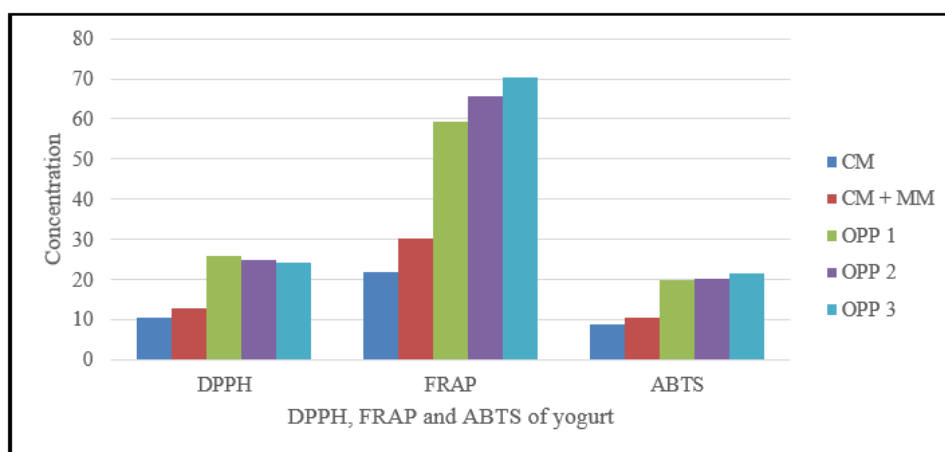
CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPP1: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder.

**Fig 3: Total phenolic content and Flavonoid content of prepared yogurt with millet milk and orange peel powder**

### 3.5. Determination of FRAP, ABTS and DPPH Radical Scavenging Activity

FRAP, ABTS and DPPH radical scavenging activity of the yogurt was examined to determine their antioxidant activity (Figure 4). This FRAP assay has been reported to be suitable to measure antioxidant activity of substances having half-reaction redox potential below 0.7 V. This measures only non-protein antioxidant capacity. Milk component such as urate, ascorbate, f-tocopherol and bilirubin have been characterized to have ferric reducing ability.<sup>42</sup> The ferric reducing capacity of each yoghurt type is shown in the figure. The total antioxidant of tested yoghurts ranged from  $21.81 \pm 0.45$  to  $70.26 \pm 0.54$  mM Fe (II)/100 g of yoghurt. Research result showed the lowest ABTS present CM variation  $8.81 \pm 0.29$  and highest ABTS present in OPP3 variation  $21.65 \pm 0.5$ . The DPPH

method was mainly used to evaluate the free-radical scavenging activity of natural antioxidant. Yogurt supplemented with barnyard millet milk and orange peel powder had considerably higher antioxidant activity (24.27% to 25.87%) than CM (10.56%) and CM + MM (12.80%). Yogurt's DPPH radical scavenging activity rose in direct proportion to increasing orange peel powder concentration. Herbal yogurts and yogurts added with sour cheery pulp were also found to have stronger antioxidant activity than plain yogurt<sup>1,43</sup>. In comparison to all the yogurt samples, orange peel powder and millet milk incorporated yogurt found high amount of antioxidant. Among the three yoghurt types OPP3 yoghurt showed the highest in total antioxidant capacity followed by CM and CM+MM. The control yoghurt showed significantly ( $p < 0.05$ ) lowest antioxidant capacity.



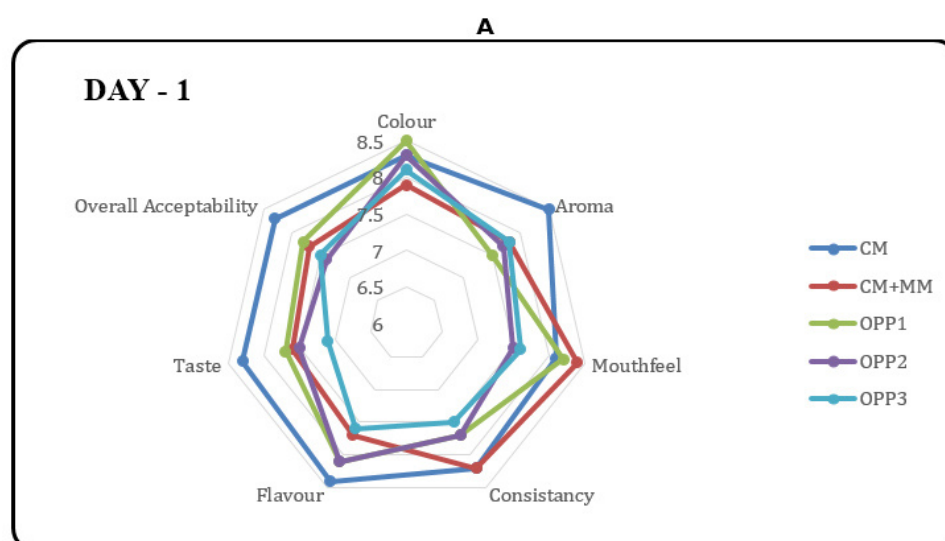
CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPP1: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder.

**Fig 4: DPPH, FRAP and ABTS of prepared yogurt with millet milk and orange peel powder**

### 3.6. Organoleptic Evaluation

The figure (figure 5) depicts the results from the sensory evaluations of the yogurt samples during 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day. On day one (figure 5 A) the OPP1 sample had the highest color, with an 8.50 value. The aroma in the CM sample was 8.50, while CM+MM and OPP3 sample showed 7.80. In CM+MM (8.40) and OPP1 (8.20) samples mouthfeel scored the highest. The consistency, flavor, and taste score for the CM sample were 8.20, 8.40 and 8.30, respectively. Overall, the CM sample had the highest value of 8.30, followed by OPP1 with 7.80, CM+MM with 7.70, and OPP2 and OPP3 with 7.40 and 7.50, respectively. OPP1 had the highest consumer acceptability among the orange peel powder and barnyard millet milk incorporated yogurt samples. Sensory evaluation of day 7 (figure 5 B) result showed CM variation overall acceptability was 8.18 and lowest overall acceptability obtained variation OPP3. Consistency obtained 8.33 in the variation CM, mouthfeel varying from 7.47 to 8.20, the flavor ranged from 7.12 to 8.22. The characteristic flavor of yogurt is due to lactic acid, which has no odor of its own, and to trace amounts of acetaldehyde, diacetyl, and acetic acid. On Day 14 (figure 5 C) each variations consumer acceptability vary. The highest value

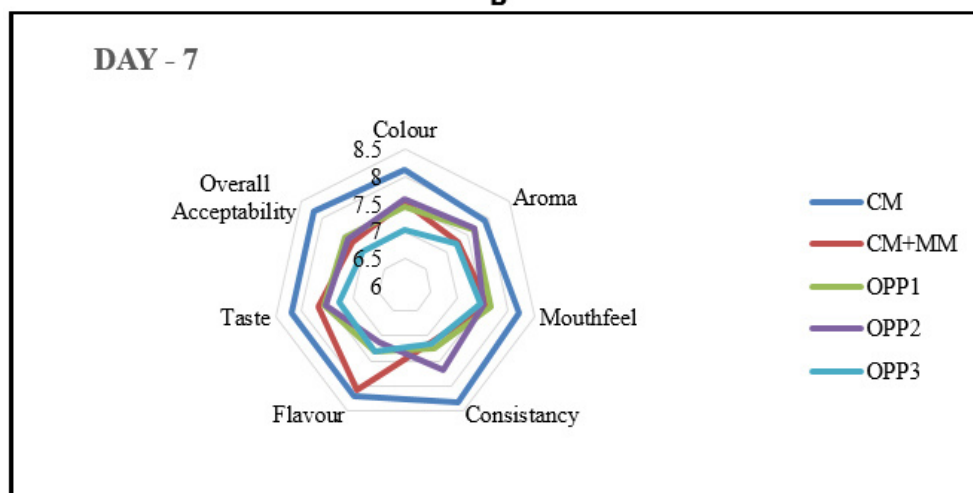
of overall acceptability was 8.03 obtained by CM and lowest overall acceptability was obtained by OPP3. The lowest color changes obtained variation was OPP2. The taste was high in CM and lowest in OPP2 with 8.16 and 7.10, respectively. A high-quality yogurt with a pleasant taste depends very much on the ratio of two bacterial species: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The streptococcus: lactobacillus ratio in the final product should be 1:1 for optimum results<sup>44</sup>. The sensory evaluation of the 21<sup>st</sup> day (figure 5 D) for different variations OPP1 obtained good consistency and overall acceptability was highly obtained OPP1 (7.37) and CM (7.27). The lowest aroma obtained CM+MM variable. The lowest Flavor obtained (5.51) OPP2 variation. Yogurt quality is particularly difficult to standardize because of the many forms, varieties, manufacturing methods, ingredients, and consumer preferences that exist. The typical yogurt flavor can only be detected in plain yogurt<sup>45</sup>. Day 28<sup>th</sup> (figure 5 E) obtained CM overall acceptability was 6.85 and OPP1 was highly accepted by the consumers after 28 days was 7.11. the taste was OPP1 variation was obtained 6.73. The result obtained some taste difference in all variations. Bitterness in yogurt is mainly due to peptides caused by the proteolytic activity of *L.bulgaricus* during storage.<sup>46</sup>



CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPP1: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder.

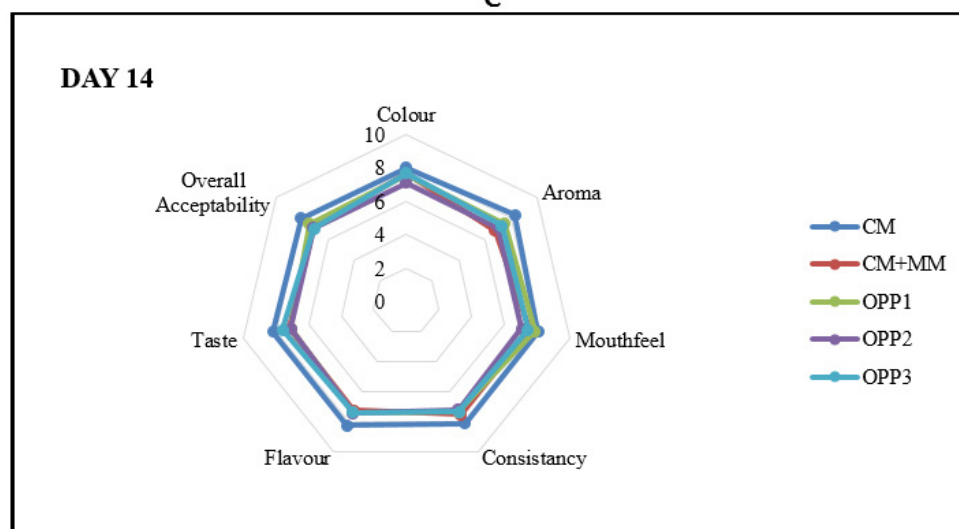


**B**



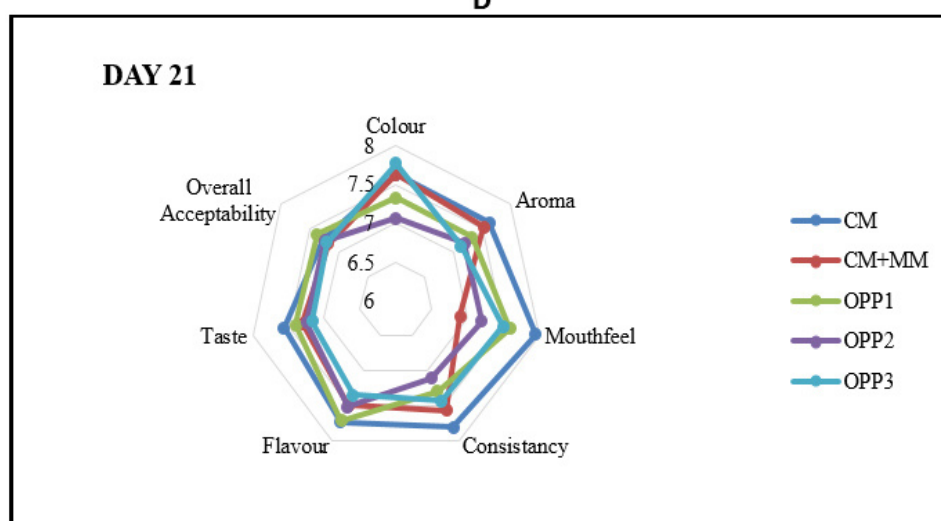
**CM:** Plain yogurt (cow's milk); **CM+MM:** Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); **OPP1:** CM+MM with 0.5% incorporation of orange peel powder; **OPP2:** CM+MM with 1% incorporation of orange peel powder; **OPP3:** CM+MM with 1.5% incorporation of orange peel powder.

**C**

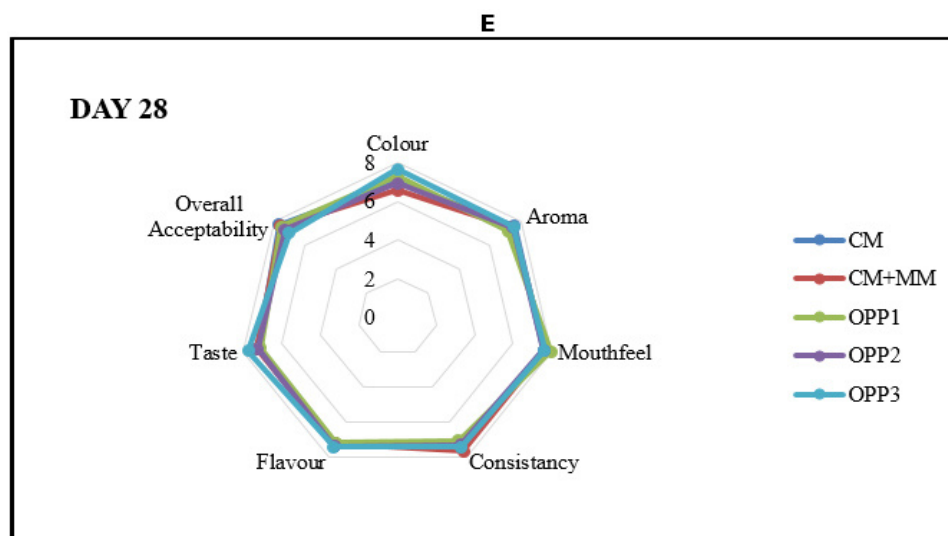


**CM:** Plain yogurt (cow's milk); **CM+MM:** Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); **OPP1:** CM+MM with 0.5% incorporation of orange peel powder; **OPP2:** CM+MM with 1% incorporation of orange peel powder; **OPP3:** CM+MM with 1.5% incorporation of orange peel powder.

**D**



**CM:** Plain yogurt (cow's milk); **CM+MM:** Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); **OPP1:** CM+MM with 0.5% incorporation of orange peel powder; **OPP2:** CM+MM with 1% incorporation of orange peel powder; **OPP3:** CM+MM with 1.5% incorporation of orange peel powder.



*CM: Plain yogurt (cow's milk); CM+MM: Cow's milk and Barnyard millet milk incorporated yogurt (1:1 ratio); OPP1: CM+MM with 0.5% incorporation of orange peel powder; OPP2: CM+MM with 1% incorporation of orange peel powder; OPP3: CM+MM with 1.5% incorporation of orange peel powder.*

**Fig 5: Graphical representation of descriptive sensory analysis of prepared yogurt with millet milk and orange peel powder. A: 1<sup>st</sup> day of storage, B: 7<sup>th</sup> day of storage, C: 14<sup>th</sup> day of storage, D: 21<sup>st</sup> day of storage and E: 28<sup>th</sup> day of storage**

#### 4. CONCLUSION

The current role of yogurt in the diet is one of the more successful and yet contentious issues in the entire food marketplace. Yogurt enjoys considerable market share in the overall diet of many parts of the world, and yet consumers have little understanding of its value to their health. For this research work scientifically proved. Yogurt, with its role of delivering live bacteria, does not fall within either of these simple categories. It is therefore not surprising that there is not yet any scientific consensus on the benefits of yogurt and the presence/abundance of live bacteria beyond its traditional role of providing essential nutrients in a dairy product to those with lactose intolerance. Thus, despite considerable evidence that yogurt as a food product is beneficial to health, its scientific evidence portfolio, regulatory position, and consumer perception remain underappreciated. An increase in

orange peel powder concentration enhanced antioxidant activity. Antioxidant activity was higher in yogurt enriched with barnyard millet milk and orange peel powder (0.5, 1 and 1.5%) than in CM and CM+MM yogurt samples. It was observed that the incorporation of orange peel powder increased the antioxidant activity of yogurt with cow's milk and millet milk.

#### 5. AUTHORS CONTRIBUTION STATEMENT

P.N helped supervise the project and SN carried out the experiment. All authors discussed the results and contributed to the final manuscript

#### 6. CONFLICT OF INTEREST

Conflict of interest declared none.

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